III. Prophylactical problems

DRUG RESISTANCE AND BETA-LACTAMASE PRODUCTION OF STRAINS OF E. COLI, PROTEUS, KLEBSIELLA AND CITROBAKTER OF DIFFERENT ORIGIN

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Key-words: drug resistance — bacterial strains — antibiotics — urinary tract infections — enteral infections

Drug resistance of Enterobacteriaceae proved also in strains isolated in the pre-antibiotic era (4) is nowadays widespread and presents a serious problem for the chemotherapy. Antimicrobial drug application on a large scale in human and veterinary medicine and in animal husbandry as well forms a selective medium for maintenance and spread of resistant bacterial strains. Selective pressure is particularly strong in urine where antimicrobial drugs excreted by the kidneys during treatment can reach rather high concentrations.

In the present work some results from investigations of the distribution of drug resistant strains belonging to the family Enterobacteriaceae which are important for the current infectious pathology are reported.

Material and methods

A total of 1286 bacterial strains were tested. They were divided according to their origin into the following groups: E. coli from: patients with urinary tract infections (I), with other non-enteral infections (II), asymptomatic bacteriuria (III), enteropathogenic strains (IV), strains from feces of healthy individuals (control group) (V). The strains of the species Proteus were isolated from patients with urinary tract infections (I), other non-enteral infections (II) and from healthy individuals (III). The strains of both species Klebsiella and Citrobacter were isolated from patients with urinary tract infections (I) and from healthy persons (II).

In order to establish to what extent the application of a given preparation influences upon the distribution of bacterial strains resistant to it we investigated the resistancy to chemotherapeutic drugs intensively or more restrictedly administered in therapeutic practice.

Minimal inhibitory concentrations (MICs) of 25 antimicrobial drugs towards the strains studied were determined by using of the method of serial dilutions in Mueller-Hinton's agar. On the basis of MIC rates some curves were elaborated reflecting cumulative percentages of the strains inhibited. Besides ß-lactamase production according to Masuda's et al. method and conjugation transfer of R-plasmids to a standard recipiend strain of E. coli K12 was also assessed.

Results and discussion

The results from the aforedescribed investigations were analysed according to the following aspects:

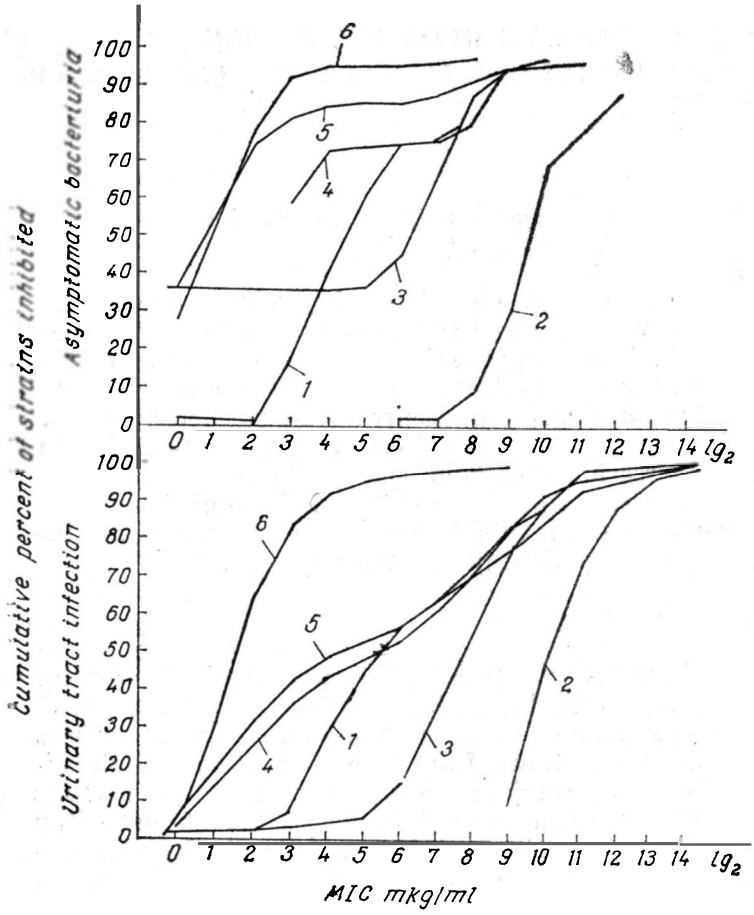


Fig. 1. Resistance against beta-lactam antibiotics of strains of E. coli isolated from patients with urinary tract infection and asymptomatic bacteriuria
1 — Benzylpenicillin; 2 — Methicillin; 3 — Oxacillin; 4 — Ampicillin; 5 — Carbenicillin; 6 — Cephalothin

1. The dependence of the drug resistance on the taxonomic belonging, period, geographic region and source of isolation of the strains.
2. The production of ß-lactamases against penicillins and cephalosporins according to the origin of the strains.
3. Correlation between drug resistance to β-lactams and β-lactamase production.

Our results showed a very high incidence of resistant and even predominantly polyresistant strains in all the bacterial groups (fig. 1-4) and enabled to emphasize some peculiarities. Polymixines M and E (colimycin), e. g., possessed a good antibacterial effect against E. coli, Klebsiella and Citobacter but a very weak one on bacteria of Proteus: even at concentrations of 16.384 mkg/ml they

inhibited 48 and 70 per cent, respectively, of the strains only. Strains belonging to the species Proteus were considerably more resistant to terizidone — a preparation used for the treatment of urinary tract infections, too (fig. 3).

On the background of high resistance to most drugs, the good antibacterial effect of rifampicin, nitrofuran, trimethoprim and 5-nitrox attracted our atten-

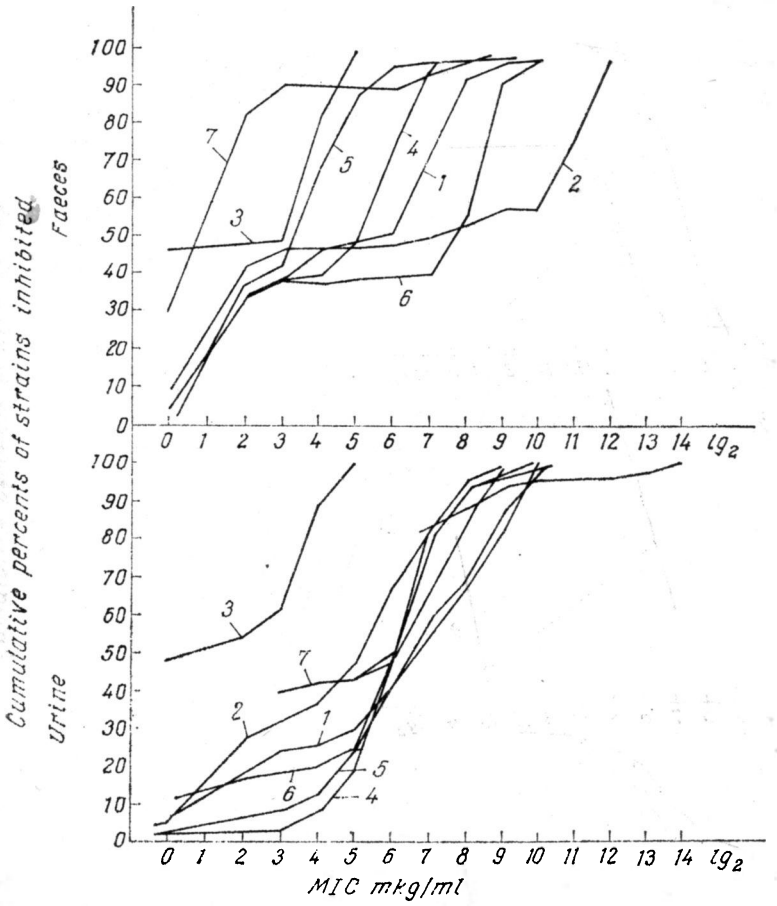


Fig. 2. Resistance against aminoglycoside and tetracyclin antibiotics of strains of Citrobacter from different sources
1 — Streptomycin; 2 — Kanamycin; 3 — Gentamycin; 4 — Tetracyclin; 5 — Doxycyclin; 6 — Methacyclin; 7 — Tetraolean

tion. Only strains, isolated from patients with urinary tract infections (fig. 3-4) demonstrated resistance to trimethoprim and co-trimoxazol. Other authors (3, 10) reported analogous data noting that in England the incidence of resistance to trimethoprim had increased when uropathogenic bacteria were concerned due to genetical determination by R-plasmids and transposons.

Some species differences of drug resistance were also ascertained: strains of the species C. freundii were more sensitive to ampicillin, carbenicillin and cephalosporins as compared with these of the species C. diversus while C. intermedius occupied a borderline position.

The comparative analysis of uropathogenic strains E. coli isolated in 1972 and 7 years later — an 1979, revealed an increased resistance to antibiotics intensively used in clinical practice during this period (ampicillin, chloramphenicol, gentamycin) and, on the contrary, a decreased one to more rarely used antibiotics (streptomycin, kanamycin) (2).

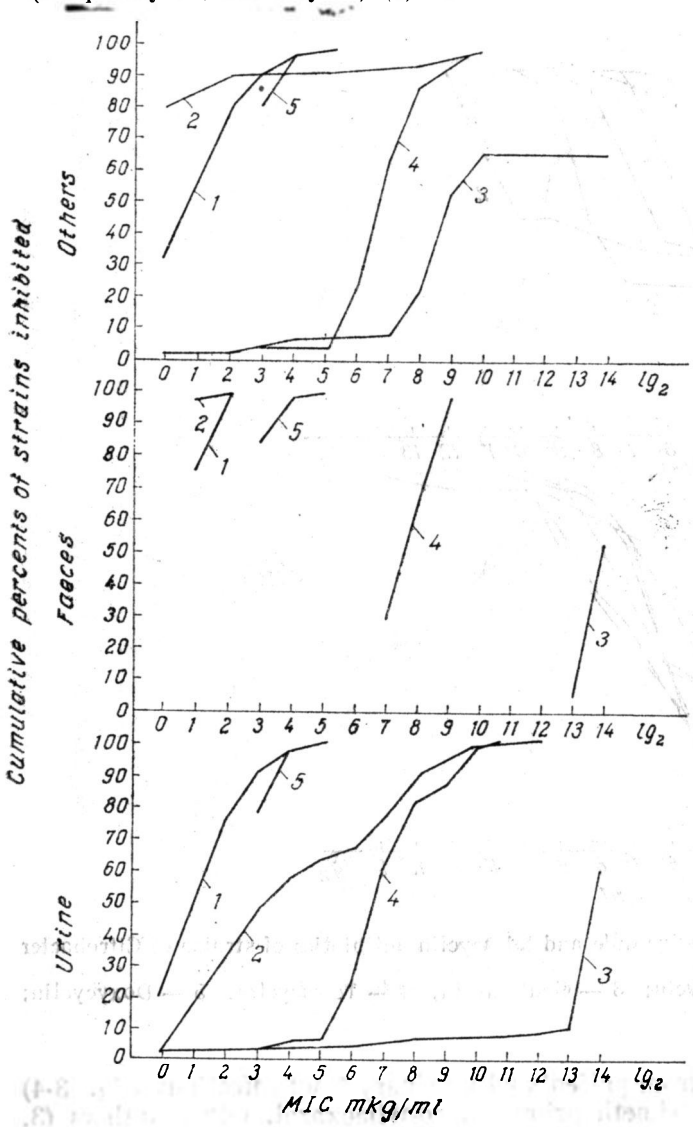


Fig. 3. Resistance against trimethoprim, co-trimoxazol, polymyxin M, terizidone and 5-nitrox of strains of Proteus from different sources
1 — Trimethoprim; 2 — Co-Trimoxazol; 3 — Polymyxin M; 4 — Terizidone; 5 — 5-Nitrox

In a considerable part of the results statistically significant differences of the resistance in relation to the source of strain isolation were established: in all the bacteria uropathogenic strains demonstrated higher resistance than the strains of another origin (flg 1-4, table 1). Enteropathogenic E. coli resistance was also lower than that of uropathogenic strains.

The higher resistance of the uropathogenic strains could be explained with the high urine concentrations of antimicrobial drugs during the treatment of the urinary tract infections and with the consecutive selection of resistant strains.

Independently of the fact that strains from healthy individuals were relatively less resistant, their resistancy considered on its own was also high. The

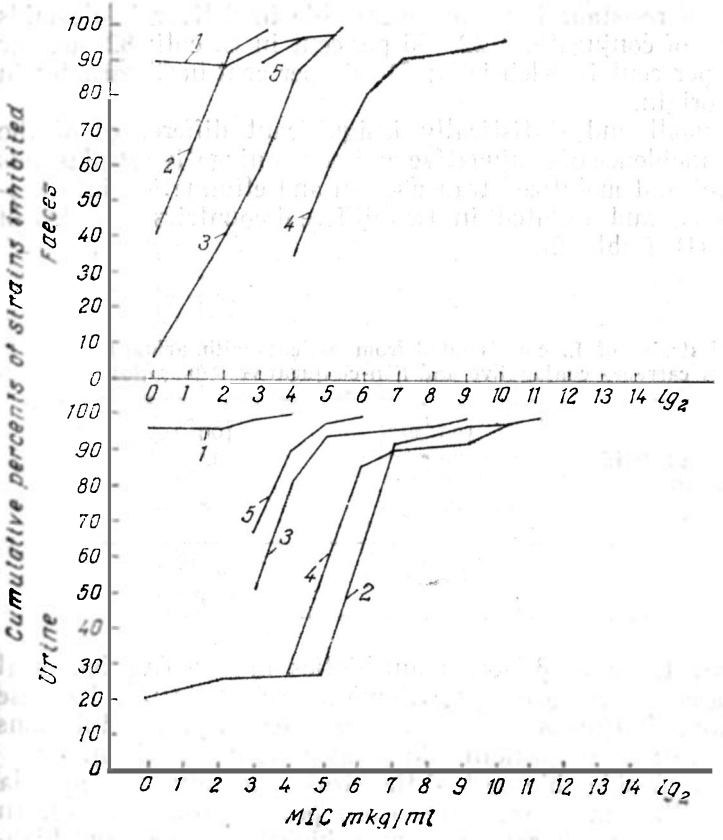


Fig. 4. Resistance against trimethoprim, co-trimoxazol, polymyxin M, terizidone and 5-nitrox of strains of Citrobacter from urine and faeces
1 — Trimethoprim; 2 — Co-Trimoxazol; 3 — Polymyxin M; 4 — Terizidone; 5 — 5-Nitrox

Table 1

Resistance against one or more chemotherapeutic drugs of strains of E. coli isolated from patients with urinary tract infection and asymptomatic bacteriuria

Sources of strains	Investigated strains	Resistant strains (n, %)	Number of chemotherapeutic drugs									
			1	2	3	4	5	6	7	8	9	10
Urinary tract infection	400	373	38	42	52	58	84	47	39	8	4	1
		93.2	9.5	10.5	13	14.5	21	11.7	9.7	2	1	0.2
Asymptomatic bacteriuria	83	41	14	12	3	2	7	3	—	—	—	—
		49.4	16.9	14.5	3.6	2.4	8.4	3.6				

frequent presence of resistant Enterobacteriaceae in healthy persons could be explained with the following reasons: 1. Permanent use of antibiotics in animal husbandry and their passage into the food (milk, eggs, meat, etc.). 2. Passing of resistant strains from individuals treated with antibiotics into untreated persons.

A considerable part of resistant isolations were able to deliver R-plasmids of R 678 strain by means of conjugation: 22—36 per cent in E. coli; 32—64 per cent in Proteus; 36—64 per cent in Klebsiella; 24—64 per cent in Citrobacter in dependence on strain origin.

There were rather small and statistically insignificant differences of the level of resistance and of incidence of conjugative and non-conjugative R-plasmids proved by means of direct and mobilized transmission and elimination into uropathogenic E. coli strains, and isolated in two different countries — Bulgaria and the Soviet Union (1) (table 2).

Table 2

Frequencies of strains of E. coli isolated from patients with urinary tract infections carrying conjugative and non-conjugative R-plasmids

Number of strains	100	100
Direct transfer of R-plasmids	26	32
Mobilizing conjugation	28	29
Elimination	10	8
total	64	69

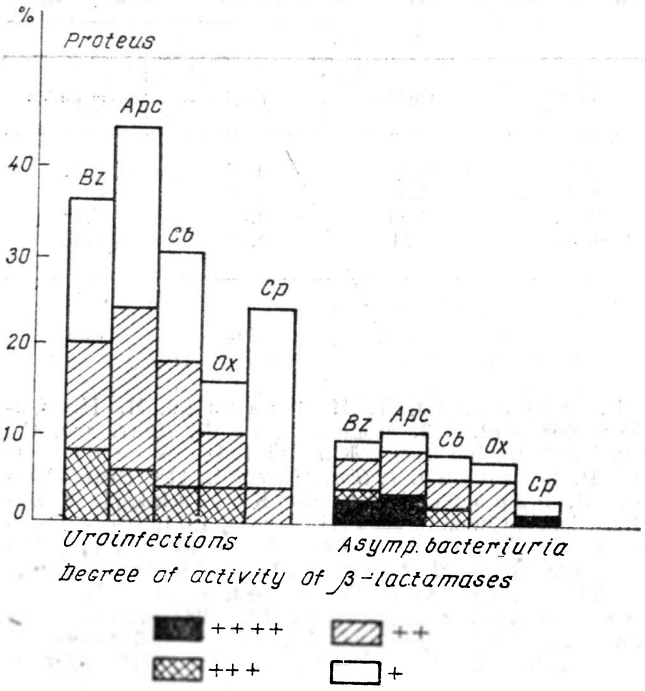
It is known that resistance to β-lactam antibiotics in sensitive bacterial population can be elevated by using of the following mechanisms: 1. Increase of β-lactamase production. 2. Changes of cellular permeability. 3. Mutations leading to structural alterations of penicillin-binding proteins (6, 7, 8).

We established that a considerable part of the strains, mainly of Klebsiella and Citrobacter isolated with urinary tract infections produce ß-lactamases. In general, uropathogenic strains are characterized by a higher resistance and higher frequency of β-lactamase production (fig. 5). However, the correlation analysis reveals a direct but not strongly expressed relation between MIC rates and β-lactamase production. Correlation coefficients are higher with Klebsiella and Citrobacter but lower ones — with E. coli (table 3).

An explanation of the event observed — lack of complete correlation between drug resistance and ß-lactamase production — can be found in the structural peculiarities of the cellular membrane of Gram-negative bacteria, in the presence of a permeability barrier between antibiotics and enzymes inactivating them (7). It is known that Gram-negative bacteria (as distinction from Gram-positive ones) possess an outer membrane that presents an important diffusion barrier against various soluble substances, including antibiotics, too, and determines the so-called «intrinsic» drug resistance of these bacteria so often observed in the practice (9).

Recently, H. Nikaido and T. Nakae (6) proved the presence in the outer membrane of a special class of proteins, called «porins» performing the function of diffusion small channels. Most antibiotics, including also ß-lactam ones, penetrate into the bacterial cell through the wide channels of the outer membrane. Even when the antibiotic is completely resistant to enzymatic hydrolysis, the

absence of wide channels can ensure survival of the bacterial cell — the large, hydrophobic and often negatively-loaded ß-lactam molecule cannot pass through the narrower channels through which vitally important sugars and amino acids do.



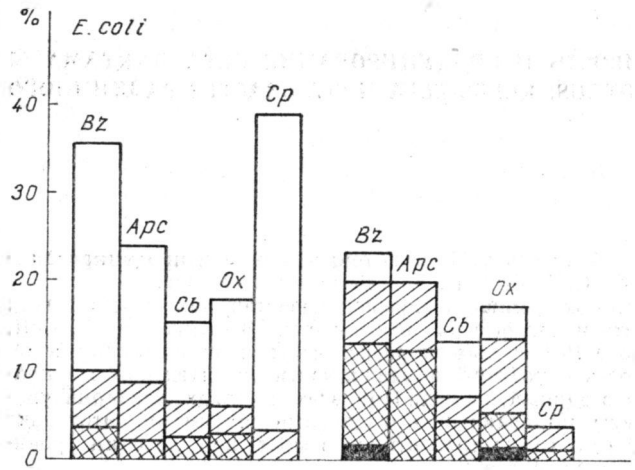


Fig. 5. Beta-lactamase in strains of E. coli and Proteus isolated from patients with urinary tract infection or asymptomatic bacteriuria

In our investigations we often observed resistance to ß-lactam with high activity of corresponding ß-lactamase. This fact can be explained by the inhibitory effect exerted by some ß-lactam antibiotics on the enzymes inactivating them.

Table 3

Correlation coefficients between the values of the minimally inhibiting concentrations of β-lactam antibiotics and production of β-lactamases in some Gram-negative bacteria

Bacteria	Antibiotics				
	benzylpenicillin	oxacillin	ampicillin	carbenicillin	cephalothin
E. coli	0.42	0.39	**0.58**	0.30	0.47
Proteus	0.56	0.41	**0.56**	0.60	0.47
Klebsiella	0.67	0.62	**0.69**	0.52	0.24
Citrobacter	0.58	0.64	0.71	0.74	0.33

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ЛЕКАРСТВЕННАЯ УСТОЙЧИВОСТЬ И ПРОДУЦИРОВАНИЕ БЕТА ЛАКТАМАЗЫ ПРИ ШТАММАХ E. COLI, PROTEUS, KLEBSIELLA И CITROBACTER РАЗЛИЧНОГО ПРОИСХОЖДЕНИЯ

Р. Маринова

РЕЗЮМЕ

Исследована лекарственная устойчивость к 25 химиотерапевтикам и продуцирование бета лактазамы при 1286 штаммах E. Coli, Proteus, Klebsiella и Citrobacter.

Полученные результаты доказывают доминирование полирезистентных штаммов всех групп бактерий и позволяют выявить некоторые их особенности. В отличие от E. Coli, Klebsiella и Citrobacter, бактерии рода Proteus высокорезистентны к полимиксину и теризидону. Лекарственная устойчивость находится в зависимости от интенсивности применения антимикробного препарата в данный период. При всех группах бактерий уропатогенные штаммы более устойчивы по сравнению с другими штаммами. Этот факт объясняется высокими концентрациями химиотерапевтиков в моче при лечении уроинфекции и селекции высокоустойчивых мутантов.

Значительная часть устойчивых штаммов являются носителями конъюгативных R плазмидов. Не установлено статистически значимых различий в уровне устойчивости и в частоте конъюгативных и неконъюгативных R плазмидов, доказанных посредством прямой и мобилизованной передачи и элиминации при уропатогенных штаммах E. Coli, изолированных у нас и в СССР.

Большая часть исследуемых штаммов, и прежде всего уропатогенные штаммы, являются продуцентами бета лактамазы. При корреляционном анализе установлена прямая, но не сильно выраженная зависимость между стоимостями минимальной ингибирующей концентрации и продуцированием бета лактамазы. Этот факт объясняется особенностями строения стенки грамотрицательных бактерий.